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ON GOLD AS A STAINING AGENT FOR NERVE TISSUES.

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TWO methods of gold staining are in use for hardened tissues. One consists in bringing the section to be stained into a dilute solution of palladium chloride, where it remains for five minutes; then into an acid solution of chloride of gold, where it remains for twenty-four hours. The myeline sheaths of the coarser fibres are stained violet.

The other method, introduced by Freud of Vienna, is used with tissues hardened in Müller's fluid. It consists in bringing the section to be stained into a one per cent. solution of chloride of gold, where it remains for from four to six hours, then successively into a twenty per cent. solution of caustic soda, and a ten per cent. solution of potassium iodide. In this last solution the section takes, in five or ten minutes, a reddish or violet color. In a successful specimen ganglion cells and axis cylinders are sharply stained, as are often also the myeline sheaths. According to some authorities the result is sometimes an axis cylinder, sometimes a myeline sheath stain. The stain results from a reduction of the gold salt to the form of an oxide, or to metallic gold. The defect of the method is its extreme unreliability, which renders it worthless for staining sections in series.

But reduction of gold chloride may be effected with great certainty by a number of reagents. They must, however, be employed with some care, in order to determine the formation of the purple or red oxide chiefly or entirely in the nervous elements of the tissues.

The following method, if faithfully carried out, will give sufficiently uniform results.

The piece of tissue from which sections are to be cut, is hardened in Müller's fluid for from two to five months; it is then washed for a few minutes in water, is brought for a day or two into fifty per cent., then into ninety-five per cent. alcohol, where it should remain for two months or longer, until it has taken a greenish tinge. It is then imbedded in celloidin and the sections cut. The sections should remain in eighty per cent. alcohol for a time varying from a few days to several weeks, before staining.

The section to be stained is brought from water into a one per cent. aqueous solution of gold chloride, where it remains for from ten to thirty minutes. It is then washed superficially in water, brought for half a minute into a ten per cent. solution of sodium hydrate, washed again, and brought into the following solution, which is called the reducing fluid, where in a few moments it takes a vivid red color.

Sulphurous acid, - - - -	5 c.c.
Tincture of iodine 5 per cent., - -	gtt. v x.
Solution of ferric chloride 37 per cent. (officinal),	gtt. i.

The section is then washed in water, and mounted in Canada balsam by the usual manipulation.

Sections, until they are brought into the reducing fluid, should be handled with platinum or some non-metallic substance, as an iron needle streaks the specimen.

The reducing fluid should always be made fresh just before using.

The stain which is taken by a specimen handled as above, varies with the time which has elapsed since the cutting of the section. If the section is stained at once,

there is a more or less diffuse stain, which, if the tissues are not too thoroughly impregnated with the bichromate, differentiates in the course of a week or more after the specimen has been mounted on a slide. This probably takes place better in the dark. The axis cylinders, and ganglion cells and their processes, are sharply stained, and the myeline sheaths are somewhat stained. At the same time there is a good deal of color in the other structures, notably the connective tissue nuclei. After a day or two in the dilute alcohol, sections take a stain which is lighter, and almost entirely confined to ganglion cells, axis cylinders and myeline sheaths. Still later ganglion cells stain little or not at all; after this the larger axis cylinders remain unstained, and a specimen is obtained in which only myeline sheaths, and the fine axis cylinders of the gray matter are stained; sections of this kind closely resemble those stained by the Weigert method; at last only myeline sheaths are capable of taking the coloring matter.

The sequence of events in the preceding method is as follows: The potassium bichromate of the Müller's fluid is reduced in the tissue to brown chromium dioxide, or to green chromic oxide, partly by the reducing action of the fresh tissue itself, partly by the alcohol into which it afterwards comes. This process is completed by the action of alcohol and light on the cut section. The oxides of chromium are displaced by the gold salt, which is then converted into the trihydroxide by the sodium hydrate, and into the red oxide by the reducing fluid.

Dilute alcohol acts on chrome salts not only as a reducing agent, but as a solvent; to this fact is due the comparatively rapid change in the manner of reaction of the sections to the gold salt, when they are kept in eighty per cent. alcohol. To prevent this change, and at the same time obtain a better differentiation of the nerve elements, the following method should be employed:

The sections, hardened as above, are brought immediately after cutting into a mixture of absolute alcohol four parts and glycerine one part. In this solution in the dark the change described above takes place little more slowly.

They may then,if necessary, be washed out for a day or two in water before staining. They are then brought for a day or two into a one per cent. solution of nitric acid in ninety five per cent. alcohol; then from water into a one per cent. chloride of gold solution, to which has been added one per cent. of nitric acid; after remaining in this last for an hour or two, they are brought successively into the soda solution and the reducing fluid as before.

The stain attained is of a bright red color, axis cylinders and ganglion cells and their processes being very distinct, myeline sheaths and connective tissue nuclei lightly tinged. The color may turn to blue or purple on keeping. This is especially apt to be the case if the section is brought into an alkaline solution just before treatment with the gold salt.

The success of the above method depends almost wholly on the hardening of the tissue. Too long a stay in Müller's fluid makes necessary a more thorough removal of the bichromate by a long stay in alcohol; the time allowed varies somewhat with the temperature, which last should be rather under eighty degrees F. than over that point; the tissue should be moderately firm, and of a decided brown color, not blackish, when removed from the Müller's fluid; when this is the case, a clear nerve fibre stain may be obtained by the use of the nitric acid solution, even after a minimum stay in alcohol.

In hardening the tissue, the bichromate may be reduced more rapidly and completely by other means than by alcohol. Harden the tissue in Müller's fluid for two or three months, then bring for a few hours into sulphurous acid. The tissue soon takes a light green color; it should then come into fifty per cent., then into ninety-five per cent. alcohol, and should remain for a few weeks in the latter; it may then be imbedded, and sections cut and stained as above. Although more rapid, this manipulation will probably not be found as advantageous as the other one.

Sections stained by chloride of gold show the great preponderance in the gray matter of the spinal cord, especially in the cervical and lumbar enlargements, of naked

over medullated nerve fibres. The larger axis cylinders are surrounded by rings of color, the most distinct one generally corresponding to the outer limit of the myeline sheath. The color in the axis cylinders and ganglion cells has the appearance of being due to a deposition of the metal in the tissue itself, as the cells are readily seen to be lying in their lymph spaces, and the nuclei and nucleoli are differentiated.

The black reaction in the Golgi stain, as shown by Rossbach and Sehrwald (Centralblatt f. die med. Wissenschaft, Juni, 1888), is due to the formation of bichromate of silver in the lymph spaces around the ganglion cells and axis cylinders, and gives the rounded outline of these spaces, not that of the cells.

In sections stained by the Weigert method there are often seen rings of color similar to those of the gold stain, which correspond to the clefts and outer surface of the myeline sheaths. This suggests the probability that the numerous smaller fibres in the gray matter, which are stained by the Weigert method, are naked axis cylinders, the color being due to a deposition of the mordant (chrome salt) around the fibre.

It might be interesting to note in connection with the mechanical theory of mordants, how much of the color in tissue staining is due to mordanting or coloring matter deposited in spaces left by nature, or by unequal contraction in hardening. Undoubted examples of space staining are seen in the demonstration of endothelial cells and of Ranvier's crosses by means of nitrate of silver, and of the corneal cells by chloride of gold. All of the myeline sheath stains, except perhaps Weigert's acid fuchsine stain, are open to this interpretation.

I have seen sections of spinal cord stained by the Weigert method, and imperfectly decolorized, in which the whole thickness of the peripheral horn-spongy tissue was occupied by a black network. This was evidently caused by coloring matter deposited in interstices due to the hardening process. The black network in liver stained by the Weigert method, seen by some observers, may be explained in the same way.